similar to that of PDGF, however, CTGF is the product of a gene completely unrelated to the A or B chain genes of PDGF. In addition, CTGF is not produced by macrophages. Since CTGF is produced by endothelial and fibroblastic cells, both of which are present at the site of a wound, it is probable that CTGF functions as a growth factor in wound healing. Pathologically, CTGF may be involved in diseases in which there is an overgrowth of connective tissue cells, such as cancer, fibrotic diseases and atherosclerosis.

The primary biological activity of CTGF polypeptide is its mitogenicity, or ability to stimulate target cells to proliferate. The ultimate result of this mitogenic activity *in vivo*, is the growth of target tissue. CTGF also possesses chemotactic activity, which is the chemically induced movement of cells as a result of interaction with particular molecules. Thus, the CTGF of this invention is mitogenic and chemotactic for connective tissue cells, however, other cell types may be responsive to CTGF polypeptide as well.

The CTGF polypeptide of the invention is characterized by existing as a monomer of approximately 36-38 kD molecular weight which is secreted by cells and is active upon interaction with a PDGF receptor on target cells. Although CTGF is antigenically related to PDGF, there is little if any peptide sequence homology.

Applicants successfully isolated and purified CTGF protein and cloned the nucleotide sequence encoding CTGF, both of which were previously unknown in the prior art.

THE REJECTIONS OF 35 U.S.C. §112

The specification is objected to under 35 U.S.C. §112, first paragraph, as failing to adequately teach how to make and/or use the invention, i.e., failing to provide an enabling disclosure. Claims 14-16 are rejected under 35 U.S.C. §112, first paragraph, for the reasons set forth in the objection to the specification. Applicant respectfully traverses these bases for rejection.

The specification is objected to as allegedly not providing enablement for the production of antibodies, either monoclonal or polyclonal, that react with the protein of the invention or fragments thereof, but do not cross-react with platelet derived growth factor (PDGF).

Given the present techniques that are available today, the production of polyclonal and monoclonal antibodies is routine. An example of one approach to producing antibodies is provided in the specification on page 9, lines 10-20. These antibodies are specifically reactive (bind) with CTGF polypeptide or fragments thereof. In addition, two references, Kohler, et al. and Ausubel, et al., are provided on page 9 as further sources to aid in antibody production. Lines 18-20 specifically state that antibodies reactive with CTGF, but not PDGF, can be identified by screening culture supernatants. It is a matter of routine to screen antibody-containing supernatants to identify those samples which contain antibodies to CTGF, but not PDGF. One skilled in the art would recognize that various assays are available to distinguish antibodies which bind PDGF from those that specifically bind CTGF. For example, one could pass a sample containing antibodies prepared against CTGF over a column with PDGF bound to it, in order to eliminate any PDGF-binding antibodies. The

non-bound fraction could then be passed over a column containing CTGF and any antibody which binds is CTGF-specific.

The Office Action places emphasis on the lack of enablement in *producing* CTGF specific antibodies, however, the key step to distinguish PDGF from CTGF antibodies is the screening process. One skilled in the art, having possession of the unique polypeptide of the invention, could easily distinguish between CTGF and PDGF reactive antibodies using routine screening methods which are generally described in the two cited references as described above. Thus, by starting with CTGF and following the teachings of the specification, or the vast number of teachings readily available in the prior art, one skilled in the art could produce, identify and isolate antibodies, monoclonal or polyclonal, which react with CTGF and not PDGF. Applicants respectfully request that this basis of rejection be withdrawn, with respect to both the specification and claims 14-16, directed to the CTGF antibodies.

Claims 1-4 and 14-16 stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Office Action states that the protein of the invention is inadequately identified in the claims. In order to avoid confusion with other proteins, it is asserted that the claimed protein should be identified by various common protein characterizations such as molecular weight, pI, amino acid sequence information, monomer or multimer, function or activity, etc. Applicants respectfully traverse this basis of rejection, but believes that the amendments made herein address the concerns raised by the Examiner.

Claim 1, as amended, now includes unique properties associated with the polypeptide of the invention, i.e., that the polypeptide and

fragments of the polypeptide which are biologically active, are mitogenic and chemotactic with respect to connective tissue cells. This property is not possessed by any other polypeptide known to Applicants.

The Office Action states that the term "functional fragments" renders claim 1 indefinite for failing to a) define what is meant by functional, and b) failing to specify what fragments are encompassed by the claim. Page 5, lines 9-15 of the specification recites that "CTGF polypeptide includes functional fragments of the polypeptide, so long as the mitogenic and chemotactic activities of CTGF are retained. Smaller peptides containing the biological activity of CTGF are included in the invention." Clearly, the biological activity, mitogenicity and chemotactic ability, is what "functional" refers to. One skilled in the art would recognize the term "functional" accordingly. Further, Example 2, pages 17-19 of the specification, provides a functional assay for CTGF activity which can be used routinely to evaluate a CTGF fragment to determine whether the fragment has the requisite biological activity.

Additionally, the Office action states that claim 14 is indefinite for failing to define "specifically reactive", and further is indefinite for citing "fragments thereof". Page 9, lines 10-20, and more specifically lines 18-20, describes what is meant as "specifically reactive". One skilled in the art would recognize that the term "specifically" refers to antibodies immunoreactive with CTGF, but not PDGF. Claim 14 has been amended above to read "bind" rather than "reactive", in order to clarify what is meant by reactive. "Fragments thereof" refers to any portion of CTGF polypeptide which contains an epitope which binds an antibody that identifies CTGF. Therefore, an antibody may bind a fragment of CTGF, but will still identify the entire CTGF polypeptide.

Antibodies do not bind to the entire 36-38 kD molecule, but only specific regions which contain antigenic sites. Therefore, whether the antibody recognizes these sites in the presence of the entire polynucleotide or just as fragments of the polynucleotide, such an antibody is included in the invention. Applicants respectfully request that this basis for rejection be withdrawn.

REJECTIONS BASED ON PRIOR ART

Claims 1-4 stand rejected under 35 U.S.C. §102 (b) as anticipated by, or, in the alternative, under 35 U.S.C. §103 as obvious over Matsuoka et al., or alternatively Campochiaro et al., or alternatively Shimokado et al. Applicants respectfully traverse these bases for rejection.

Matsuoka et al. disclose a family of PDGF-related proteins found in human wound fluid. These proteins were identified using a polyclonal antiserum to PDGF. Two peptide fractions, from 16-17 kD and 34-36 kD, were found to be immunoreactive with the polyclonal antibodies. The Office Action states that the 34-36 kD protein appears to be identical to the CTGF of the present application.

The Applicants would like to emphasize that the antibodies used for "purification" in the Matsuoka reference were polyclonal which, because of their inherent non-specificity, reacted with a broad family of biologically distinct proteins. Therefore, only a *family* of PDGF-related proteins were purified by Matsuoka. The individual members of the family were not purified away from each other and therefore the actual purity of the peptide fraction 34-36 kD is highly questionable. In contrast, the present invention is clearly distinguishable in teaching a "substantially pure" CTGF which is isolated from other proteins with which it naturally occurs.

Additionally, the mitogenic and chemotactic activities in Matsouka correlated only with the 16-17 kD peptide(s), as shown in figure 4. The 34-36 kD fraction did not possess any biological activity and was only present in trace amounts at the time when PDGF-like bioactivity is observed in wound fluid. Matsouka discloses, at best, an undefined family of antigenically related proteins that cross react with a polyclonal sera to PDGF. In view of the failings of Matsouka to teach or suggest the polypeptide of the invention, Applicants respectfully request that the rejections based on this reference be withdrawn.

Campochiaro, et al., disclose the isolation of a PDGF-like protein from retinal pigment epithelial cells. Western blot analysis using polyclonal anti-PDGF antiserum identified an 18.5 kD band and a band of 36-38 kD in retinal pigment epithelial cell conditioned media. Using a anti-PDGF IgG affinity column, bands with molecular weights of just less than 36 kD and 18 kD were found.

The proteins disclosed by Campochiaro, et al., were identified using polyclonal antiserum to PDGF and therefore a family of PDGF-related proteins was identified. The individual proteins were not purified. On page 221, line 1, the authors state that they found "multiple bands of 36-38 kD" and a band at 18.5 kD, which possessed mitogenic and chemotactic activities. This confirms that the antiserum identified a family of proteins. There is no data that the biological activity observed was specifically due to the 36-38 kD peptide. The activity may have been due to another protein which was not even detected by Western blot analysis. On pages 225-226, in the last paragraph of the discussion, the authors state that "PDGF-like proteins from different cell types may have structural differences that account for differences in migration, but retain important functional and antigenic similarities that warrant combining them under the heading of PDGF-like proteins."

Therefore, the 36-38 kD protein(s) disclosed by Campochiaro, et al., cannot be characterized as anything other than additional members of the PDGF-related protein family. Consequently, the Applicants respectfully request that any rejections based on these references be withdrawn.

Shimkado, et al. discloses the isolation of a PDGF-like protein having a molecule of 37 kD, isolated from activated human alveolar and peritoneal macrophages. Two classes of PDGF-like proteins which contained mitogenic activity and were antigenically similar to PDGF were between 37-39 kD and 12-17 kD. Page 281, column 1, lines 1-10 show that the mitogenic activity of the 14-17 kD fraction and the 37 kD fraction represent approximately 56 and 40 % of the mitogenic activity in the macrophage conditioned medium. The figure legend to figure 6 states that the recovery is only 15-20% of the total activity loaded on the gel. Therefore, it is difficult to conclusively state how much activity is actually present in any one fraction and how much is attributable to one specific protein. Additionally, on the same page, lines 14-43, under non-reducing conditions, alveolar macrophages contain several PDGF-related proteins. Using polyclonal antisera, it would be expected to identify several members of the PDGF-like family, however, it is not possible to say that a secreted protein(s) found on a gel in a region corresponding to molecular weight 37-39 kD is one protein and that that protein is the CTGF of the present invention. The Applicants respectfully request that any rejections based on these references be withdrawn.

Further, without amino acid or nucleic acid sequence data, one cannot assume <u>a priori</u> that a single gene encodes the protein described in Matsouka et al., Campochiaro, et al., or Shimkado, et al., and that that gene also encodes the CTGF of the present invention. In the case of <u>Amgen Inc. v. Chugai Pharmaceutical Co.</u>

Ltd. (CAFC 1991) 927 F2d 1200, 18 PQ2d 1016), the court stated that "conception has not been achieved until reduction to practice has occurred, i.e., until after the gene has been isolated." Thus, since there is no disclosure of identical or closely homologous sequences between the proteins in the cited references and the CTGF of the invention, let alone isolation of a gene, there is no indication that any of the proteins are one in the same. The Applicants respectfully request that any rejections based on these references be withdrawn.

Claims 1-4 stand rejected under 35 U.S.C. §102(a) as anticipated by or, in the alternative, under 35 U.S.C. §103 as obvious over Ryseck.

The CTGF of the present invention was cloned and sequenced prior to the May 1991 disclosure by Rysek. Applicants submitted the CTGF cDNA sequence in confidence to Genbank on July 17, 1990, and made the sequence publicly available through Genbank in December, 1991. Therefore, regardless of whatever teachings may be present in Rysek, the present invention clearly predates Rysek, and any rejection based on this reference should be withdrawn. If the Examiner would prefer, Applicants will submit an affidavit attesting to the invention of the gene sequence of CTGF which predates the Rysek reference.

Claims 14-16 stand rejected under 35 U.S.C. §103 as being unpatentable over Matsouka et al., or alternatively Campochiaro, et al., or alternatively Shimokado et al., or alternatively Rysek et al. as cited in the rejections under 35 U.S.C. 102/103 above.

The Office Action states that it would have been obvious to one of ordinary skill in the art to generate antibodies specific to the

growth factor and non-crossreactive with similar species for numerous reasons.

As stated in the comments above, the CTGF of the present invention and the proteins identified in the above-cited references are distinguishable. In other words, antibodies which specifically bind CTGF could not have been produced prior to Applicants discovery of the molecules which makes CTGF available for immunization. As stated above, the references fail to teach or disclose CTGF as defined herein. Therefore, since the CTGF of the invention is a novel protein, antibodies which specifically bind to CTGF must also be novel. There would not have been any motivation to combine any of the above-cited references to produce antibodies, since they teach other PDGF-related proteins, not CTGF. The antibodies used for isolating the proteins in the above-cited references were raised against PDGF and not CTGF. The antibodies are not specific for CTGF as they recognize other growth factors including PDGF AA, AB and BB and leukocyte derived growth factor (LDGF). Therefore, the antibodies of the invention which bind CTGF and not PDGF are novel. The Applicants respectfully request that any rejections based on these references be withdrawn.

In summary, based on Applicants' amendments to the claims and comments above, it is respectfully submitted that claims 1, 4 and 14-16 clearly and patentably define the invention. Applicants respectfully request that the Examiner reconsider the various grounds of rejection set forth in the Office Action and, in light of Applicants response, allow the claims now pending to proceed to issuance.

If the Examiner would like to discuss any of the issues raised in the Office Action, Applicants representative would welcome the opportunity. Applicants representative can be reached at (619) 455-5100.

Respectfully submitted,

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